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5/2/2005
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TOXICITY AND FATE OF TWO MUNITIONS CONSTITUENTS IN SPIKED SEDIMENT EXPOSURES WITH THE MARINE AMPHIPOD *EOHAUSTORIUS ESTUARIUS*

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Abstract—The lethal toxicity of the explosive compounds ^{14}C-labeled 2,4,6-trinitrotoluene (TNT) and nonradiolabeled hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the estuarine amphipod *Eohaustorius estuarius* was investigated in 10-d spiked sediment exposures. The 10-d median lethal concentration (LC50) was determined using the sum molar initial concentration of TNT, aminodinitrotoluenes (ADNTs), and diaminonitrotoluenes (DANTs), as determined by high-performance liquid chromatography (HPLC), and collectively referred to as HPLC-TNT*. Despite expectations of higher toxicity in sandy sediment (Yaquina Bay [YB], OR, USA) compared to relatively fine-grained sediment (San Diego Bay [SDB], CA, USA), LC50 values were similar: 159 and 125 µmol/kg, for YB and SDB sediments, respectively. When expressed as the sum of TNT and all its degradation products (^{14}C-TNT*), LC50s were approximately two times the corresponding LC50s determined by HPLC. The HPLC-TNT* fraction likely corresponds to the most bioavailable and toxic transformation products. The concentrations of ^{14}C-TNT* in tissues were substantially higher than those for HPLC-TNT*, suggesting that compounds other than TNT and its major aminated transformation products were prevalent. Critical body residues were similar for exposures to SDB (11.7 µmol/kg) and YB sediments (39.4 µmol/kg), despite marked differences in the nature of compounds available for uptake in the exposure media. The critical body residues for *E. estuarius* are lower than those reported for other aquatic invertebrates (83–172 µmol/kg). Unlike observations for TNT, RDX was only loosely associated with SDB sediment, with near complete recovery of the parent compound by chemical analysis. Exposure to RDX did not result in significant mortality even at the highest measured sediment concentration of 10,800 µmol/kg dry weight, nor tissue concentrations as high as 96 µmol/kg wet weight. The lack of RDX lethal effects in this study is consistent with results reported for other invertebrate species.

Keywords—2,4,6-Trinitrotoluene   Hexahydro-1,3,5-trinitro-1,3,5-triazine   *Eohaustorius estuarius*   Critical body residue

INTRODUCTION

Explosive compounds were released extensively to the environment during manufacturing, handling, and disposal operations at numerous military sites in the United States and throughout the world, resulting in contamination of surface and groundwaters, soils, and sediments [1,2]. An additional source of explosives contaminants is present as unexploded ordnance and dumped ammunition in marine and estuarine environments [3,4]. Common explosives such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and their degradation products previously have been observed to be recalcitrant in many environmental matrices, leading to long-term contamination of military sites where they were released [5]. The nitroaromatic explosive TNT was the most-abundantly produced explosive manufactured worldwide over the last century. Its release to surface and groundwater mostly is from runoff and leaching from storage and disposal areas, as well as release from receiving lagoons at munitions production and processing facilities [2]. In water, degradation products of TNT primarily are formed through photolysis [6]. Reduction of the nitro- groups to amino- groups for TNT has been observed in organically rich waters and soils [5]. The major aminated metabolites of TNT are aminodinitrotoluenes (2- or 4-ADNT) and diaminonitrotoluene (2,4- or 2,6-DANT). The military-unique explosive RDX, a cyclonitramine, is one

of the most powerful and common conventional munitions presently used [7]. This explosive has been released into waste streams generated during manufacture and processing, leaching from storage lagoons and burial areas into groundwater, and demilitarization operations [2]. The explosives RDX and HMX have lower sorption coefficients in topsoil compared to TNT and are found more commonly in groundwater [8].

Explosives and related compounds are known to cause a variety of organism-level effects in aquatic receptors, as reviewed by Talmage et al. [2] and reported in recent studies [9–18]. Therefore, the presence of such compounds in aquatic environments may cause detrimental impacts to animal and plant populations and, consequently, is a significant assessment and remediation challenge.

Little effort has been devoted to investigating the toxicity of explosives toward potential marine and estuarine biological receptors, unlike the numerous toxicological investigations using freshwater species (reviewed in Talmage et al. [2]; see also [9,10,14,18]). Aqueous toxicity data have been generated for only a few species of marine invertebrates and fish [11,15–17,19].

Reports on the toxicity of explosive and related compounds in sediment exposures are also scarce [9,10,13,15,17,18]. The relationship between whole-sediment chemical concentrations and biological effects were reported using TNT-spiked sediments in laboratory exposures for the marine amphipod *Leptocheirus plumulosus* and the polychaete *Neanthes arenaceodentata* exposed to TNT [13], 2,4-DANT, trinitrobenzene, RDX, and HMX [15]. Similar studies have been conducted

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for the marine amphipod *Ampelisca abdita* exposed to 2,6-dinitrotoluene, tetryl, and picric acid [17], for the freshwater amphipod *Hyalella azteca* and *Chironomus tentans* exposed to TNT, 2,4-DANT, trinitrobenzene, RDX, and HMX [20], and for the freshwater oligochaete *Tubifex tubifex* exposed to TNT [10]. In addition, the pore water toxicity of sediments spiked with 2,6-dinitrotoluene, tetryl, and picric acid were investigated for the marine invertebrates *A. abdita* and *Dinophilus gyrociliatus* (polychaete), and spores of the macroalgae *Ulva fasciata* [17].

The primary objective of the present study was to evaluate the toxicological effects of TNT and RDX to the estuarine amphipod *Eohaustorius estuarius* in sediment exposures. In addition, the use of two sediment types (fine-grained and sandy) allowed for comparison of the fate and bioaccumulation of TNT under contrasting conditions. Exposed organisms were evaluated for the presence of explosives and metabolites in their tissues. Due to the tendency for TNT to degrade rapidly and bind to sediment [10] and tissues [20], radioactivity was used in combination with traditional chemical analysis to better trace the fate of TNT in both sediment and tissues. Toxicity was related to chemical concentrations in each exposure matrix and organism to derive toxicity metrics for potential use in ecological risk assessments of explosives and unexploded ordnance contamination in marine environments.

METHODS AND MATERIALS

Chemicals

Radiolabeled trinitrotoluene ([^{14}C]TNT, 23.6 Ci/mol) was purchased from New England Nuclear Research Products (Boston, MA, USA). Manufacturer-reported radiochemical purity was 99%. Nonradiolabeled TNT (99% pure) was purchased from Chem Service (Westchester, PA, USA). Purified RDX (>98% pure) was obtained from the Naval Surface Warfare Center (Indian Head, MD, USA). The molecular mass is 227 g/mol for TNT and 222 g/mol for RDX; water solubility is 573 μmol/L for TNT and 173 μmol/L for RDX; and log K_{ow} is 1.60 for TNT and 0.87 for RDX [2].

Sediments

Uncontaminated surficial sediments from San Diego Bay (SDB), California, USA (total solids content = 67%; silt and clay content = 23%; total organic carbon content = 0.36%) and Yaquina Bay (YB), Oregon, USA were stored in the dark at 4°C for approximately one week before spiking. Contaminant concentrations were below effects range low [21] for both sediments. The YB sediment had no detectable organic carbon; thus, it was combined with a small amount (6.4% on a dry wt basis) of fine-grained clean sediment from Sequim Bay (WA, USA) to simulate coarse-grained sediment with low total organic carbon content. The modified YB sediment was characterized by a total solids content of 75%, a silt and clay content of 5%, and a total organic carbon content of 0.09%.

Spiking procedure

A separate 5-ml dosing solution was prepared for spiking each sediment at target sediment concentrations (Table 1). Solutions consisted of a constant amount of [^{14}C] TNT (19.2 and 22.8 μCi, for the YB and SDB sediments, respectively, targeting a concentration of 5 × 10^4 dpm/g sediment dry wt) and the appropriate amount of TNT dissolved in acetone required to obtain the respective target concentrations of nonradiolabeled TNT in sediment. Control dosing solutions contained 5

Table 1. Target sediment concentrations and measured sum-molar concentrations of 2,4,6-trinitrotoluene (TNT) and TNT degradation products as determined using liquid scintillation counting (LSC) and high-performance liquid chromatography (HPLC). Sampling was conducted immediately following the 4-h mixing period (spike), and on days 0 and 10. YB = Yaquina Bay (Newport, OR, USA); SDB = San Diego Bay (San Diego, CA, USA). Values in parentheses are standard deviations. (n = 3 for sediment LSC measurements, n = 1 for HPLC and all other LSC measurements)

Sediment	Target mg/kg dry wt	Target μmol/kg dry wt	Sediment LSC Spike	Sediment LSC Day 0	Sediment LSC Day 10	Sediment HPLC Spike	Sediment HPLC Day 0	Sediment HPLC Day 10	Overlying water LSC Day 0	Overlying water LSC Day 10	Overlying water HPLC Day 0	Overlying water HPLC Day 10	Pore water LSC Day 0	Pore water LSC Day 10	Pore water HPLC Day 0	Pore water HPLC Day 10
YB	6	28	30 (1)	24 (1)	21 (3)	13	8	0	0.5	1.2	0	0	7.8	2.6	0.0	0.0
	12.5	55	51 (2)	40 (2)	32 (1)	34	19	8	1.3	2.7	0.7	0.9	17.9	5.5	6.6	1.8
	25	110	105 (3)	83 (2)	71 (3)	79	49	19	2.6	6.6	1.7	2.1	49.6	13.8	26.1	7.5
	50	220	163 (1)	106 (2)	87 (7)	161	107	47	6.6	13.0	6.4	9	98.5	25.1	93.7	22.2
	100	440	426 (35)	280 (13)	176 (20)	278	194	79	11.5	37.8	6.1	22.4	310.0	72.1	221.8	48.0
SDB	25	110	112 (4)	46 (3)	68 (9)	103	0	0	2.4	2.8	1.0	1.1	43.4	3.2	11.7	0.0
	50	220	324 (28)	128 (4)	95 (29)	227	30	0	8.7	18.0	4.8	7.9	188.2	19.6	81.5	7.0
	100	440	461 (26)	259 (4)	116 (9)	405	116	14	16.5	41.8	12.4	31.0	326.4	40.9	223.1	25.7
	200	880	734 (57)	325 (65)	180 (16)	652	209	41	19.4	16.0	7.9	52.7	288.9	92.0	235.6	73.4
	400	1,760	1,521 (30)	1,057 (119)	622 (49)	1,539	967	298	31.8	88.9	25.9	68.2	379.7	281.1	299.2	250.8

ml of acetone without TNT. The specific activity (dpm/μmol) of the isotopically diluted [^{14}C] TNT in each spiking solution was determined by measuring radioactivity (dpm/ml) by liquid scintillation counting (LSC), and TNT concentration (μmol/ml) with high-performance liquid chromatography (HPLC) analyses. Radioactivity measured in the exposure media (sediment, pore water, overlying water) and tissues were expressed as molar equivalents of TNT (μmol/kg) by dividing the measured radioactivity by the specific activity derived from the appropriate dosing solution. Solid (crystallized) RDX was added directly to sediment to create different treatments. Therefore, dosing solutions were not prepared for this chemical.

For the TNT experiments, each dosing solution was added to 30 g of clean, dry quartz sand contained in an aluminum weigh boat under a fume hood. After evaporation of the solvent (~30 min), the TNT-coated sand was added to a 4-L beaker containing 1.5 kg of sediment. Sediment and sand then were mixed vigorously for 4 h with a laboratory impeller mixer (Lightnin, Avon, NY, USA). Using this procedure, TNT was spiked into SDB sediment at target concentrations of 0, 110, 220, 440, 880, 1,760 μmol/kg (25, 50, 100, 200, and 400 mg/kg), on a dry weight basis. Correspondingly, target TNT concentrations for the YB sediment were 0, 28, 55, 110, 220, and 440 μmol/kg (6.25, 12.5, 25, 50, and 100 mg/kg). Target concentrations for both sediments were identical when normalized by the organic carbon content of the sediment (30.6, 61.1, 122.2, 244.4, 488.9 mol/kg organic carbon). Concentrations were selected based on previous experiments using the amphipod *L. plumulosus* [13]. Following mixing, a sample of each spiked sediment was collected and analyzed as described below.

The compound RDX was added directly without solvent to the SDB sediment, because unrealistically large quantities of solvent (up to 60 ml/kg sediment) would have been required to dissolve RDX to the desired concentrations. Sediment and solid RDX were mixed in the same manner as the sediment and TNT-coated sand in TNT experiments. Target concentrations were 0, 675, 1,350, 2,700, 5,400, and 10,800 μmol/kg (150, 300, 600, 1,200, and 2,400 mg/kg). Following mixing, a sample of each spiked sediment was collected and analyzed as described below. Care was taken to ensure that all sediment processing was conducted in near darkness to prevent photolytic degradation, which has been reported as a primary degradation mechanism for TNT and RDX [2].

Experimental animals

Eohaustorius estuarius is an estuarine, deposit-feeding amphipod tolerant of a wide range of sediment characteristics, yet is demonstrably sensitive to contamination [22]. Animals were collected by Northwest Aquatics (Newport, OR, USA) from a clean field site near YB and shipped overnight in collection-site sediment to the Engineering Research and Development Center (Vicksburg, MS, USA). Amphipods were shipped at salinities between 7 and 10□ and were acclimated slowly to the 20□ experimental salinity by water exchange, resulting in an increase of approximately 3□ /d. During the holding period, animals were provided a thin layer of their home sediment, received trickle-□ow aeration, and were kept at a constant 15°C. Amphipods used for the TNT and RDX exposures ranged in size from 3 to 5 mm.

Amphipod 10-d experiment

Toxicity experiments were conducted following U.S. Environmental Protection Agency guidance [22]. Exposures were conducted in 1-L glass beakers containing 200 g of spiked sediment and 800 ml of 20□ synthetic seawater (Crystal Sea® Marinemix, Marine Enterprises International, Baltimore, MD, USA). Five replicate beakers per concentration were used for the TNT exposure in SDB sediment; the TNT exposure with YB sediment employed four replicates; and the RDX exposure consisted of three replicates. Fewer replicates were used in the YB-TNT and RDX experiments because of insufficient availability of field-collected amphipods. An additional replicate from each treatment for each exposure was sampled destructively on day 0 for measurement of sediment, overlying water, and pore water chemical concentrations. Twenty amphipods were added to each beaker on day 0, approximately 24 h after sediment addition to beakers and 48 h after the initial spike. Beakers were provided trickle-□ow aeration to maintain dissolved oxygen levels and covered with watch glasses to suppress evaporation. No food was added throughout the exposures. All experiments were static, nonrenewal exposures, as recommended by Conder et al. [10] for explosive-spiked toxicity exposures. The experiments were illuminated constantly using gold □uorescent bulbs (λ > 500 nm) in order to prevent photodegradation of the nitroaromatic compounds. Beakers were held in a recirculating water bath maintained at 15 ± 1°C. Unmodified amphipod collection-site sediment (YB) served as the negative control.

At experiment termination, the overlying water was sampled for chemical analysis and decanted carefully for final volume determination. The sediment was sampled for chemical analysis and sieved through a screen with a mesh size of 500 μm to collect and enumerate surviving amphipods. Three animals per replicate were blotted dry, weighed, and assayed for radioactivity. The remaining animals were blotted dry, weighed, and frozen at −80°C for chemical analysis.

Chemical analysis

Sediment. Sediment samples were not air-dried before extraction, as recommended by Nipper et al. [23] for related compounds, to minimize potential degradation to unknown and solvent-resistant degradation products. For each sediment sample, three subsamples (0.05–0.1 g) were assayed for radioactivity (see *Tissue* section) and one sample (5 g) was mixed vigorously with 10 ml of acetonitrile and sonicated for 18 h (Branson 3200, Branson Ultrasonics, Danbury, CT, USA) in an 18°C water bath (Neslab RTE-111, Neslab Instruments, Newington, NY, USA). The extract was recovered, filtered through polytetra□uoroethylene syringe filters (Nalge Nunc, Rochester, NY, USA) and mixed with ultrapure water on a 1:1 ratio by volume. An aliquot of the diluted extract (1 ml) was assayed for radioactivity (see *Radioactivity assay* section). Analytes in the extract were quantified (see *Analytical chemistry (HPLC)* section). Sediment moisture content was determined by the weight ratio of wet and oven-dried (55°C) triplicate samples.

Pore water and overlying water. Pore water was obtained by centrifugation of sediment at 4,000 rpm for 20 min and followed by filtering. Overlying water was sampled directly from the exposure beaker. Water samples were assayed for radioactivity (see *Radioactivity assay* section). Water samples were diluted as needed, and analytes were quantified (see *Analytical chemistry (HPLC)* section).

Tissue. For each experimental beaker, three amphipods were analyzed for radioactivity. The previously frozen remaining animals (30–60 mg) were thawed and transferred to polypro-

pylene bead-beater vials. Each vial received 100 mg of 1-mm glass beads and 0.75 ml of HPLC-grade acetonitrile. Samples were homogenized using a miniature bead-beater (Biospec, Bartlesville, OK, USA) for 100 s at 4,200 oscillations/min and placed on ice. Samples received 0.75 ml of 1□ CaCl₂ and were sonicated for 1 h in an 18°C water bath. The sonicated samples then were centrifuged for 10 min at 7,500 *g* at 4°C, and the supernatants were filtered through 0.45-μm polytetra□uoro-ethylene syringe filters into amber sample vials. Analytes were quantified as described below (see following section).

Analytical chemistry (HPLC). The compounds TNT, 2-ADNT, 4-ADNT, DANTs (concurrent detection of 2,4-DANT and 2,6-DANT isomers), and RDX in aqueous samples and diluted solvent extracts were separated and quantified by HPLC following a modification of U.S. Environmental Protection Agency SW-486 method 8330 [24]. Analyses were conducted with an Agilent 1100 Series HPLC (Palo Alto, CA, USA) equipped with a Supelco RP-Amide C-16 column and a diode array detector. Sample injection volume was 100 μl with a □ow rate of 1 ml/min and column temperature of 45°C. An isocratic mobile phase consisting of 55□ methanol and 45□ water was used. Absorbance was measured at 230 and 254 nM. Peak identification was based on retention time with spectral analysis confirmation. The laboratory reporting limit for all analytes was 3 μmol/kg (~0.6 mg/kg) for sediment samples, 0.5 μmol/L (~0.1 mg/L) for water samples, and 5 μmol/kg (~1 mg/kg) for tissue samples. Recoveries for TNT and RDX ranged from 90 to 98□.

Radioactivity assay (LSC). Samples were placed in a xy-lene-based scintillation cocktail (3a70b, Research Product International, Mt. Prospect, IL, USA) and assayed for radioactivity on a Tri-Carb liquid scintillation analyzer (Model 2500 TR, Packard Instrument, Meridien, CT, USA). Solid samples (sediment and whole amphipods) were disrupted in the scintillation cocktail using a Branson Sonifier 450 high-intensity probe sonicator (Danbury, CT, USA).

Sediment organic carbon. Homogenized sediments were sampled before they were spiked, acidified to remove carbonates, and analyzed for organic carbon content on an Astro 2100 analyzer (□ellweger Analytics, League City, TX, USA).

Water quality

Water quality parameters were measured daily and all were within the range considered acceptable for *E. estuarius* [22]. Temperature ranged from 14.8 to 16.1°C, pH ranged from 7.6 to 8.2, dissolved oxygen ranged from 8.1 to 9.8 mg/L, total ammonia ranged from <1 to 6 mg/L, and salinity consistently remained 20□.

Statistical analysis

All measured experimental values are expressed as mean ± 1 standard deviation. For animal survival data, one-way analysis of variance was used to determine differences between means, at a 0.05 level of significance. Survival data were arcsine square-root transformed and complied with the requirement of normality and equal variance. Median lethal concentrations (LC50) and median lethal residues (LR50) values were determined using the trimmed Spearman-Karber Method. Dunnett□s method was used to compare treatment means with control means to calculate the no-observed-effect concentration, no-observed-effect residue, lowest-observed-effect concentration, and lowest-observed-effect residue.
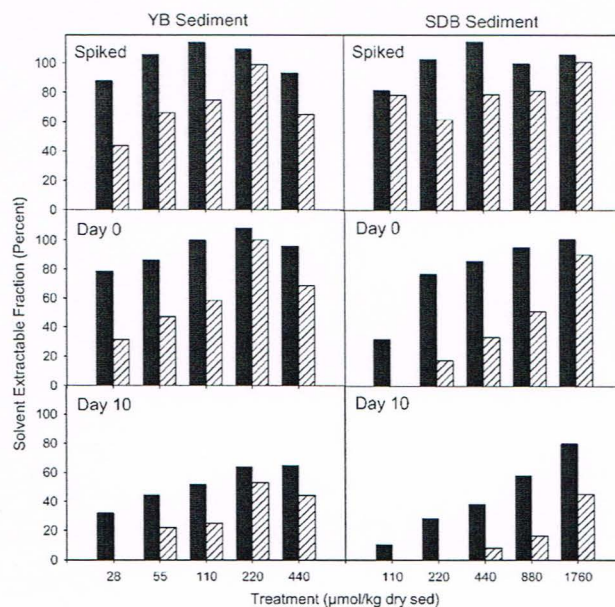

Fig. 1. Percent of total radioactivity in sediment samples corresponding to 2,4,6-trinitrotoluene (TNT) and all its degradation products, as determined by liquid scintillation counting extracts (■), and to TNT and its degradation products quantifiable by high-performance liquid chromatography of solvent extracts (▨) for toxicity exposures conducted with ¹⁴C-TNT–spiked Yaquina Bay ([YB], Newport, OR, USA) and San Diego Bay ([SDB], San Diego, CA, USA) sediments.

RESULTS

The compound TNT and all its degradation products collectively will be referred to as TNT*. The compound TNT and all its transformation products (including those chemically bound to other molecules) measured in this study using LSC will be referred to as ¹⁴C-TNT*. The compound TNT and its transformation products measured in this study using individual compound separation and quantification via HPLC (ADNTs and DANTs) will be referred to as HPLC-TNT*. Concentrations of ¹⁴C-TNT* and HPLC-TNT* are expressed as the total sum-molar concentrations (as TNT equivalents for ¹⁴C-TNT* concentrations).

Sediment chemistry

The low coefficient of variation (<9□) for triplicate samples of small sediment aliquots (50–100 mg) used for radioactivity determination indicated that the radiolabel was distributed homogeneously in the sediment following the 4-h mixing of the TNT-coated sand with the sediments for all exposure treatments. The ¹⁴C-TNT* concentrations measured after spiking and mixing closely matched target concentrations for the SDB and YB sediments (Table 1).

The fraction of TNT* corresponding to solvent-extractable TNT* in the sediment increased proportionally with the concentration for both sediments during all sampling periods, demonstrating a higher fraction of radioactive compounds resistant to solvent extraction for low concentrations (Fig. 1). The fraction of solvent-resistant transformation products in the sediment also increased with time for both sediments. However, the fraction of solvent-resistant compounds was higher in the SDB sediment. Comparison of the extract ¹⁴C-TNT* concentrations and the HPLC-TNT* concentrations in Figure 1 demonstrates that a substantial fraction of TNT* in the extract did not correspond to HPLC-TNT*. The fraction of
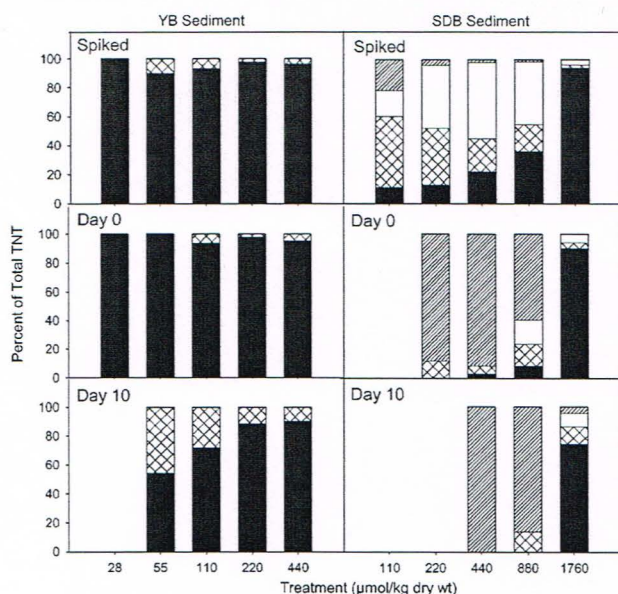
Fig. 2. Percent of total concentration in sediment samples corresponding to the concentration of trinitrotoluene (TNT; ■), 2-aminodinitrotoluene (□), 4-aminodinitrotoluene (▤), and diaminotritoluenes (▨) in solvent extracts of ^{14}C-TNT–spiked Yaquina Bay ([YB], Newport, OR, USA) and San Diego Bay ([SDB], San Diego, CA, USA) sediments used in toxicity exposures.

HPLC-TNT* in the solvent extract typically was lower in SDB sediments compared to YB sediments. Moreover, a more dramatic decrease of the HPLC-TNT* fraction during the course of the experiment occurred in SDB sediment. Because of the extensive transformation of spiked TNT in these sediments, HPLC-TNT* concentrations were lower than ^{14}C-TNT* concentrations, sometimes by a large factor (Table 1).

The TNT spiked into sediment degraded to aminated transformation products during mixing and also during the sediment exposure (Fig. 2). Different patterns of TNT degradation were observed for the two sediments. Although TNT comprised the largest fraction of HPLC-identified compounds during the experiment in YB sediment, aminated transformation products were the dominating compounds in extracts collected for most treatments of the SDB sediment (Fig. 2). The extent of TNT degradation decreased with increasing spiking concentration at all sampling times for SDB sediments, and a similar trend clearly was observed for the day 10 samples from YB sediments (Fig. 2).

Measured concentrations of RDX in spiked sediments were similar to target concentrations (Table 2). Sediment concentrations at exposure termination were lower than initial concentrations, and this concentration decrease was inversely proportional to the spiked concentration (Table 2), with virtually no change in concentration observable for the highest treatments. The presence of degradation products of RDX in sediment solvent extracts was not identifiable in the HPLC chromatograms.

Pore water and overlying water chemistry

Pore water and overlying water results are tabulated in Table 1. The TNT* concentrations in both of these water matrices increased with increasing sediment concentration for both sediments. Upon experiment initiation, the ^{14}C-TNT* and the HPLC-TNT* concentrations were substantially higher in the pore water than in the overlying water for both sediments. At experiment termination, the TNT* concentrations in the pore water had decreased, and the concentrations in the overlying water had increased above the initial concentrations. Additionally, the magnitude of the difference between the pore water and the overlying water concentrations decreased, with the overlying concentration exceeding the pore water concentration in some treatments. The LSC concentrations were higher than the HPLC concentrations in both the overlying water and the pore water for both sediments, indicating the presence of nonidentified transformation products of TNT. The relative concentration of HPLC-TNT* in the pore water and overlying water was very similar to the relative concentration of those compounds in sediment extracts for both sediment types and exposure periods (Fig. 2).

Results for RDX are tabulated in Table 2. Similar to the results observed for TNT experiments, concentrations of RDX in the overlying water increased from day 0 to day 10, although concentrations in the pore water typically decreased. Final concentrations of RDX in the pore water and overlying water increased with increasing sediment concentrations, with the concentrations in the pore water approaching the reported water solubility limit.

Spiked compound mass balance

For the TNT experiments, the total radioactivity associated with sediment particles (excluding pore water), sediment pore water, and overlying water, was determined from one replicate beaker at experiment initiation and termination using the total mass and LSC-concentration data for each compartment. Approximate masses of sediment particles, pore water, and overlying water were 134, 66, and 800 g, respectively, for the SDB sediment beakers, and 150, 50, and 800 g for the YB sediment beakers. At experiment initiation, a substantial fraction of TNT* was associated with the pore water and overlying water rather than with sediment particles, particularly for the SDB treatments (Fig. 3). Overall, the fraction of TNT* associated with the sediment particles decreased (for YB sediment) or increased moderately (SDB sediment), though the fraction associated with the pore water decreased substantially for both sediments at termination of the 10-d exposure (Fig. 3). The corresponding increase in the fraction associated with the overlying water compartment is consistent with the movement of a substantial fraction of TNT* in the pore water at exposure initiation to the overlying water during the exposure period. The fraction of TNT* associated with the sediment compartment decreased with increased spiking concentrations for the YB sediment, but a similar trend was not apparent for the SDB sediment (Fig. 3).

A total mass balance for TNT* at the onset of exposure indicated that a substantial portion of the initial amount of the ^{14}C-labeled TNT added to the exposure chambers as TNT-spiked sediment was not accounted for in the exposure beakers. These losses were 6 to 22□ for YB and 13 to 36□ for SDB sediments. No significant additional radioactivity losses were measured in the exposure chamber at experiment termination.

At experiment initiation, the RDX in the experiment chambers largely was associated with the sediment (91–99□). At experiment termination, the spiked compound primarily remained associated with the sediment, although the contribution of overlying water and pore water to the overall mass balance increased (Fig. 4).

Table 2. Targeted and measured hexahydro-1,3,5-trinitro-1,3,5-triazine concentrations in sediment, overlying water, and pore water samples taken on days 0 and 10 from toxicity exposures conducted with spiked San Diego Bay (San Diego, CA, USA) sediment

Target in sediment		Measured					
		Sediment (μmol/kg dry wt)		Overlying water (μmol/L)		Pore water (μmol/L)	
(mg/kg dry wt)	(μmol/kg dry wt)	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
150	675	787	272	7	19	68	30
300	1,350	1,547	1,126	8	23	88	49
600	2,700	2,488	2,056	11	39	114	70
1,200	5,400	4,906	4,849	15	56	154	99
2,400	10,800	10,040	10,153	7	85	143	151

Toxicity

Control performance was high in the two TNT experiments, with 89 ± 8 and 82 ± 8□ survival in YB and SDB sediments, respectively (Table 3). The lower survival in SDB controls may be due to the fine-grained nature of that sediment, as reported previously for this species [25], which naturally inhabits sandy sediments. Although control survival means were lower than recommended for standard toxicity testing using amphipods [22], this did not impact negatively the experiments, and the YB and SDB sediments would be considered nontoxic using minimum significant difference threshold criteria commonly used in sediment-quality investigations [26]. Mean negative control (unmodified YB sediment) survival was 98 ± 4□.

The expected increase in amphipod mortality with increasing sediment contamination level was observed for both sediments. Mean percent survival (± 1 standard deviation) and resulting toxicity metrics are listed in Tables 3 and 4, respectively. Using nominal sediment concentrations, 10-d LC50 values were 68□ higher in SDB sediment compared to YB sediment. Using sediment ^{14}C-TNT* concentrations on day 0, the SDB sediment LC50 was 42□ higher. When expressed as HPLC-TNT* concentrations, however, the converse relationship was observed, with the LC50 for YB sediment 27□ higher than that for the SDB sediment (Table 4). The LSC LC50s were 27 and 230□ higher than the HPLC LC50s for YB and SDB sediment, respectively (Table 4).
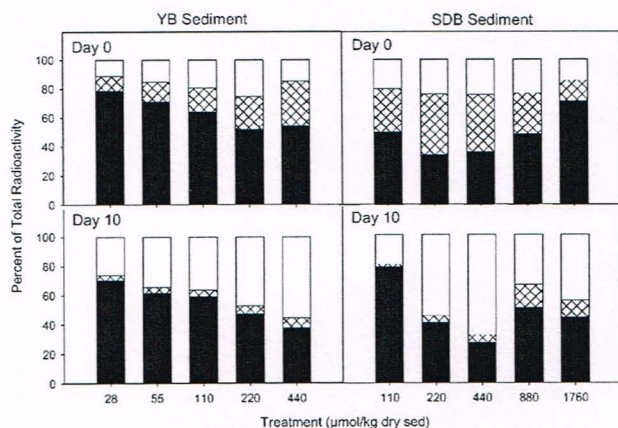
Control performance was high in the RDX experiment, with 87 ± 13□ survival. No significant mortality was observed for any exposure concentration, thus preventing the calculation of toxicity metrics (Table 3).

Bioaccumulation and critical body residues

Tissue residues increased with increasing sediment concentration and mortality for both sediments (Table 3). Toxicity metrics are listed in Table 4. The ^{14}C-TNT* concentrations in the tissues were as much as two orders of magnitude greater than HPLC-TNT* concentrations. The LSC LR50s were 45□ higher in the YB sediment exposure compared to the SDB sediment exposure. The HPLC LR50 values were 3.4-fold higher in the YB sediment exposure, as compared to the SDB sediment exposure.

Contrary to observations for sediment, the parent form of TNT was not observed in any of the tissue extracts. The compounds 2-ADNT and 4-ADNT were the only degradation products quantified in tissue extracts, with 4-ADNT representing 83 and 72□ of the total for YB and SDB sediment–exposed amphipods on average, respectively. This is consistent with the increased presence of 4-ADNT observed in the sediments. The DANTs were not measured in the tissues from either TNT exposure, in contrast with the elevated presence of DANTs in the SDB sediment.

The HPLC-tissue concentrations generally increased with increasing sediment RDX concentration (Table 3). Tissue-

Fig. 3. Percentage of the total radioactivity in the exposure chamber associated with sediment particles (■), pore water (▦), and overlying water (□) at days 0 and 10 of toxicity exposures using Yaquina Bay ([YB], Newport, OR, USA) and San Diego Bay ([SDB], San Diego, CA, USA) sediments spiked with ^{14}C-trinitrotoluene.
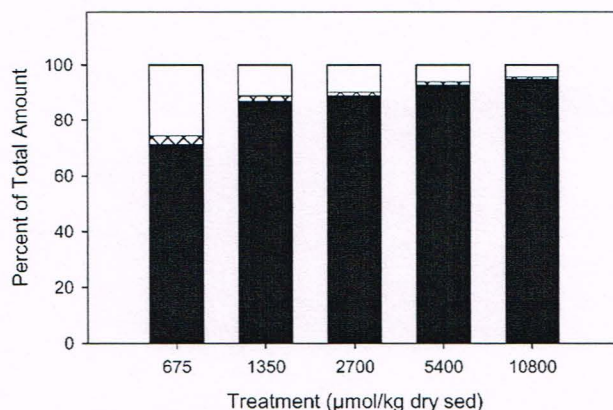
Fig. 4. Percentage of the amount of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the exposure chamber associated with sediment particles (■), pore water (▦), and overlying water (□) upon termination of toxicity exposures using RDX-spiked Yaquina Bay ([YB], Newport, OR, USA) sediment.

Table 3. Survival and body residues (mean + 1 standard error) for each treatment of the toxicity exposures conducted with Yaquina Bay ([YB], Newport, OR, USA) and San Diego Bay ([SDB], San Diego, CA, USA) sediments spiked with ^{14}C-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). — indicates no data. The asterisk (*) indicates significant difference from the control. Measurements were made by liquid scintillation counting (LSC) and high-performance liquid chromatography (HPLC), n = 4 for TNT–YB, 5 for TNT–SDB, and 3 for RDX

Compound	Sediment	Treatment (µmol/kg dry wt)	□ Survival	Body residue (µmol/kg wet wt)	
				LSC	HPLC
TNT	YB	0	89 (8)	—	—
		28	85 (4)	46 (5)	5.6 (3.8)
		55	95 (7)	98 (1)	10.2 (8.7)
		110	93 (5)	267 (5)	26.5 (3.7)
		220	80 (9)	463 (16)	43.7 (14)
		440	28 (9)	* 572 (150)	37.4 (0)
TNT	SDB	0	82 (8)	—	—
		110	88 (7)	42 (34)	0 (0)
		220	83 (8)	275 (61)	8.2 (16.0)
		440	65 (9)	367 (6)	4.9 (6.5)
		880	14 (14)	* 382 (132)	62.7 (0)
		1,760	0 (0)	* 0 (0)	0 (0)
RDX	SDB	0	87 (13)	—	—
		675	90 (0)	—	30.8 (0.9)
		1,350	88 (3)	—	25.7 (20.0)
		2,700	75 (9)	—	61.3 (48.0)
		5,400	70 (10)	—	95.9 (29.3)
		10,800	83 (20)	—	82.0 (71.3)

based toxicity metrics could not be calculated, however, due to the absence of a dose response. Observed HPLC-TNT* concentrations in tissue extracts as high as 95.9 µmol/kg did not correspond to a significant reduction in survival (Tables 3 and 4).

DISCUSSION

Fate of TNT

In the relatively silty SDB sediments, TNT aminated transformation products were the dominant compounds at termination of the short mixing period that followed spiking, except for the highest treatment where relatively little degradation initially was observed. Further degradation in the exposure beakers led to an increase in the concentration of DANTs, a substantial increase in the fraction of extractable but not HPLC-quantified compounds, and an increase in the nonextractable fraction that was very pronounced in the low treatments. In the sandy YB sediments, a similar fate for the spiked TNT was observed, but only 4-ADNT was identified by HPLC analysis. Fast degradation to aminated transformation products was reported for TNT [10,18,27] and for the related compounds trinitrobenzene [15,18] and 2,6-dinitrotoluene [17,23]. The transformation of TNT to 4-ADNT over that to 2-ADNT was favored for the SDB and YB sediments in this study, similar to that previously reported for soils [5] and sediments [10]. It has been demonstrated that this pathway is preferable thermodynamically [28]. The overall extent of TNT transformation was lower in the YB sediment than in the SDB sedi-

Table 4. Toxicity metric values for exposures conducted with Yaquina Bay ([YB], Newport, OR, USA) and San Diego Bay ([SBD], San Diego, CA, USA) sediments spiked with ^{14}C-trinitrotoluene (TNT). Toxicity metrics are based on day 0 concentrations, derived using target and measured sum-molar concentrations of TNT and TNT degradation products as determined using liquid scintillation counting (LSC) and high-performance liquid chromatography (HPLC) methods. Values in parentheses represent the 95□ confidence interval. An — indicates value not applicable. Metrics defined, LC50 = median lethal concentration; NOEC = no-observable-effects concentration; LOEC = lowest-observable-effects concentration; LR50 = median lethal residue; NOER = no-observable-effects residue; LOER = lowest-observable-effects residue

Sediment type	Method	Units	Sediment[a]			Tissue[b]		
			NOEC	LOEC	LC50	NOER	LOER	LR50
YB	Nominal	µmol/kg	220	440	348 (320–380)	—	—	—
	LSC		106	280	202 (179–228)	463	572	539 (504–567)
	HPLC		107	194	159 (147–171)	43.7	37.4	39.4 (36.1–43.0)
	Nominal	mg/kg	50	100	79 (73–86)	—	—	—
	LSC		24	64	46 (41–52)	—	—	—
	HPLC		24	44	36 (33–39)	—	—	—
SDB	Nominal	µmol/kg	440	880	586 (538–639)	—	—	—
	LSC		259	325	287 (267–308)	367	382	371 (365–377)
	HPLC		116	209	125 (96–162)	4.9	62.7	11.7 (4.7–29.1)
	Nominal	mg/kg	100	200	133 (122–145)	—	—	—
	LSC		59	74	65 (61–70)	—	—	—
	HPLC		20	38	23 (19–29)	—	—	—

[a] Dry weight basis.
[b] Wet weight basis.

ment. Similarly, degradation rates were faster in fine-grained sediments compared to sandy sediment for 2,6-dinitrotoluene [23]. The inverse relationship between spiking level and magnitude of degradation observed in this study has been reported previously for TNT and related compounds in sediments [10,15,18] and soils [29,30]. This likely resulted from increasing saturation of binding sites in the sediment particles and inhibition of microbial activity at higher concentrations of TNT and its transformation products.

The HPLC-TNT* represented only a fraction of the total amount of extractable TNT* in the sediments, and that fraction decreased during the exposure period (Fig. 1 and Table 1). Sediment concentrations of HPLC-TNT* decreased sharply during the 24-h period before exposure initiation and during the exposure period, most dramatically at the lower concentrations spiked into the SDB sediment. Likewise, nonidentified compounds represented a substantial fraction of the TNT* in the pore water and overlying water. It is important to note that these potentially bioavailable, but nonidentified, compounds associated with TNT [30] were present in exposure beakers and may contribute to observed toxicities. A large variety of molecules result from the transformation of TNT under aerobic and anaerobic conditions [30,31]. Non–HPLC-quantified compounds in the pore water and overlying water correspond to either freely dissolved TNT transformation products or to compounds bound to dissolved organic matter, as reported for TNT-spiked soils [32,33]. Because most intermediate compounds in the aerobic reductive degradation of TNT have high affinity for dissolved organic matter, most non-HPLC compounds likely were bound to that compartment. The bioavailability and toxicological significance of dissolved organic matter–bound TNT* is unknown.

The rapid disappearance of HPLC-TNT* in spiked sediments has been reported previously [10,18] and was attributed to covalent binding to sediment organic matter, as extensively reported for soils [10,33–35] and freshwater sediments [27]. By comparing the radioactivity in whole sediments with the radioactivity in sediment solvent extracts, we provide the first reported evidence for the presence of TNT*-bound residues in spiked marine sediments. Hundal et al. [30] refers to the solvent extract fraction as potentially available. Bound residues in soils following solvent acetonitrile extraction were associated with different soil organic carbon compartments such as humin, fulvic and humic acids, and lignocellulose [36]. Due to the strong nature of those bonds, nonsolvent extractable compounds are expected to be nonbioavailable to the soil biota [37], as corroborated by toxicity studies of treated soils [38,39].

Because the overlying water in the exposure beakers was not exchanged following initial loading, losses of the initial amount of ^{14}C-TNT added to beakers, as well as the relative partitioning among sediment particles, pore water, and overlying water, were determined. Loss of radioactivity following addition of sediment and overlying water to beakers indicates that some of the TNT transformation products volatilized out of the overlying water during the 24-h period between sediment and overlying water loading to the beaker and amphipod exposure initiation. The disproportionately large fraction of the radioactivity associated with the pore water at experiment initiation was not surprising given the large mass of TNT added to the sediment and the high water solubility and relatively low affinity of TNT for organic carbon. Over the course of the experiment, a fraction of the radioactivity associated in the

pore water moved to the overlying water, therefore decreasing the concentration in the sediment and pore water compartment. Substantial partitioning of spiked compounds to the overlying water of exposure beakers was reported previously for TNT [10], RDX, and HMX [15]. Exchange of overlying water during the exposure period likely would have decreased further the concentration of spiked compounds in the sediment–pore water component. Exchange of overlying water, therefore, is not recommended for sediment toxicity studies with explosives and related compounds [10] or for other weakly hydrophobic compounds.

Fate of RDX

Only SDB sediment was used for RDX toxicity exposures in this study. Exceedingly high concentrations were selected based on the absence of lethal effects of RDX-spiked sediments to the amphipod *L. plumulosus* [15]. Because nonradiolabeled RDX was used, only HPLC concentrations were measured. The concentration of RDX in the sediment declined only in the lower treatments, and the concentration decrease was inversely related to the spiking concentration, similar to the concentration decreases observed for TNT* in the same type of sediment. Sediment and pore water RDX moved to the overlying water, similar to TNT* in this study and to RDX and HMX in a previous study [15], but the relative contribution of the aqueous compartments to the overall mass balance was substantially lower for the exposures conducted in this study. To attain target concentrations in the sediment, RDX was added to sediments in excessive amounts, up to 0.24□ of the sediment dry wt. The RDX has only very low sorption affinity to particulate organic matter, as determined empirically [40–42] and as expected by its low K_{ow}. Addition of water to the beakers likely brought some of the nonsorbed fraction of the added compound into solution in the overlying water. However, the ratio of total RDX added to beakers via spiked sediment to the volume of water in the exposure beakers (113–1,810 μmol/L) exceeded the water solubility of RDX (173 μmol/L) in most treatments. Therefore, a large fraction of the spiked RDX likely remained as undissolved crystals throughout the exposure period, especially in the higher treatments. Despite this possible presence of undissolved crystals, the concentration of RDX in the pore water only remained close to the solubility limit in the highest treatment, and remained below that limit in the overlying water for all treatments. The static nature of the exposure and the low diffusion and dissolution rate of RDX [43] would explain the lower-than-expected concentrations of RDX in the pore water and overlying water during the exposure period in this study. The high recovery of RDX from sediment in this study is consistent with observations by Cataldo et al. [44], who reported >95□ extractability of RDX in soil studies that lasted 60 d, with no transformation products apparent.

TNT toxicity

In this study, the toxicity of TNT* in two sediments with different total organic carbon and grain size characteristics was compared. Toxicity was expected to be higher in YB sediments than in SDB sediment, due to its lower total organic carbon and course-grained nature. However, the lethal toxicity of both sediments was similar, as indicated by measured LC50 values. Although similar, the LSC LC50 was lower for the YB sediments, but the HPLC LC50 was higher for the YB sediments compared to SDB sediments. The lack of agreement between LC50 values likely results from the different nature of TNT

degradation products and their relative concentration in the two sediments. For example, for the 440 μmol/kg treatment, 70□ (average of day 0 and 10 measurements) of the extractable TNT* was quantifiable by HPLC in the YB sediments, though only 31□ was quantifiable by HPLC in the SDB sediment (Fig. 1). Overall, the TNT parent compound comprised a much higher fraction of the TNT* in the YB sediments compared to SDB sediments, and DANTs and unknown extractable products comprised most of the TNT* in the SDB sediments. Therefore, it is plausible that TNT transformation products are more toxic to *E. estuarius* than TNT parent compound, explaining the higher-than-expected toxicity of the more organically rich SDB sediment. Water-only studies indicate that aminated transformation products are more toxic than the parent compound in a variety of aquatic organisms [13,14]. Griest et al. [14] found 2,4-DANT to be substantially more toxic to *Cerioda-phnia dubia* than TNT or ADNTs. Davenport et al. [45] observed a higher incidence of developmental abnormalities in sea urchin larvae exposed to ADNTs compared to TNT. It is unknown whether unidentified degradation products contributed to the observed toxicity of TNT-spiked sediments to *E. estuarius* in this study. The toxicity of TNT transformation products other than ADNTs and DANTs to invertebrates has not been investigated.

In this study, point estimates were calculated based on the initial sediment concentration expressed as nominal, ^{14}C-TNT*, and HPLC-TNT* sediment concentrations. Because the nonsolvent-extractable TNT is deemed nonbioavailable due to covalent bonding with organic molecules in soils and sediments [37–39], and the chemical characterization and bioavailability of the extractable, non-HPLC TNT* remains unknown, toxicity metrics based on the HPLC concentration probably are the most appropriate, and conservative, choice for use in risk assessments.

The short sediment storage period following spiking resulted in substantial decreases in the concentration of spiked compounds and their transformation products during the exposure, which precluded the accurate determination of sediment concentrations associated with mortality, as previously observed in similar studies using spiked sediments [10,13,15,18]. Therefore, effects concentrations expressed as initial concentration are expected to have overestimated the concentrations associated with the onset of most mortality [10]. Visual inspection of exposure beakers indicated that most mortality occurred during the first 2 to 3 d of the 10-d exposure. Therefore, toxicity metrics determined using initial sediment concentrations likely were reasonable surrogates of lethal sediment concentrations in this study.

To our knowledge, no toxicity studies with explosives have been reported with *E. estuarius*. However, in TNT studies with the tube-building estuarine amphipod *L. plumulosus* using estuarine sediment with high organic carbon content (2.7□), Green et al. [13] derived a measured LSC LC50 value of 203 mg/kg (893 μmol/kg). This value is substantially higher than that observed in this study. The exposure by Green et al. [13] differed from this study in that it had a longer duration (28 d), multiple renewals of the overlying water, and a different water temperature (23°C). Therefore, it is likely that differences in the fate and bioavailability of TNT* in the two studies were substantial. In previous exposures to SDB sediments, lethal concentrations observed for *L. plumulosus* (G. Lotufo and G. Rosen, unpublished data) were similar to lethal concentrations for *E. estuarius* in this study, suggesting similar

sensitivities between the two species. The toxicity of TNT, trinitrobenzene, and 2,4-DANT also was examined using the amphipod *Hyalella azteca* exposed to spiked freshwater sediments [18]. For the TNT and 2,4-DANT exposures in the latter study, low survival (<50□) was observed at the measured initial concentration of 2,4-DANT of only 0.1 mg/kg, suggesting that *H. azteca* likely is more sensitive to TNT and its degradation products compared to marine amphipods. In addition, the HPLC LC50 value for the oligochaete *Tubifex tubifex* in 28-d exposures to spiked freshwater sediment (184 μmol/kg) [10] was very similar to the 10-d LC50 values reported in the present study. Due to the high degradation of TNT in the sediments, however, sensitivity comparisons are difficult and probably best suited to aqueous exposures with frequent renewal of exposure solutions.

RDX toxicity

The lack of mortality observed in exceedingly high concentrations of RDX is not without precedent. Lotufo et al. [15] reported no lethal toxicity for *L. plumulosus* or the marine polychaete *Neanthes arenaceodentata* upon exposure to RDX concentrations up to 1,080 mg/kg (4,565 μmol/kg), the highest concentration used. Similarly, Steevens et al. [18] did not observe any significant mortality in RDX exposures for nominal sediment concentrations as high as 250 mg/kg (1,126 μmol/kg) with the freshwater amphipod *H. azteca*. Tolerance of *E. estuarius* to RDX is further supported by lack of mortality, despite exposure to pore water concentration approaching the solubility limit of RDX in the two highest treatments. A lack of lethal toxicity to RDX for a variety of aquatic organisms also was reported by Nipper et al. [16]. In that study, significant effects on survival in seawater RDX exposures for mysid shrimp, redfish, and polychaetes (*Americamysis bahia, Sciaenops ocellatus,* and *Dinophilus gyrociliatus,* respectively) were not observed. In addition, normal fertilization and embryo development of sea urchins (*Arbacia punctulata*) were observed in saturated solutions. The absence of lethal toxicity associated with RDX-saturated aqueous solutions also has been reported for freshwater invertebrates [11,46].

Bioaccumulation and critical body residue

Despite the presence of TNT, ADNTs, and DANTs in the sediment, pore water, and overlying water in exposures to spiked SDB sediments, only 2-ADNT and 4-ADNT were identified and quantified in amphipod tissue extracts. Likewise, only ADNTs were detected in amphipods exposed to spiked YB sediments, although TNT was the most prevalent compound in the sediment and aqueous media. The nitro- groups of TNT characteristically undergo biochemical reduction in living systems [2,5]. In the exposures to YB sediments, TNT likely was metabolized efficiently to 2-ADNT and 4-ADNT. Aqueous exposure to TNT previously has resulted in the presence of ADNTs in the tissues of the aquatic invertebrates [20,47] and terrestrial invertebrates [48,49]. The apparent preferential metabolism of TNT to 4-ADNT over that to 2-ADNT in *E. estuarius* has been observed previously in other animals [47,49,50]. Although amphipods in the SDB sediments were exposed primarily to DANTs, these compounds may not have bioaccumulated at levels above the detection limit in this study, because DANTs bioconcentrate at lower levels than TNT and ADNTs in *T. tubifex* [47]. In aqueous exposures to TNT, the metabolites DANTs were not detected in *T. tubifex*, and were

detected at relatively lower levels in other aquatic invertebrates [20].

The concentrations determined in this study for HPLC-TNT* in tissues were substantially lower than those for ^{14}C-TNT*, which is consistent with determinations for sediments. Therefore, we believe that compounds other than TNT and its major aminated transformation products were present in the exposed amphipods, as previously reported for aquatic invertebrates exposed to radiolabeled TNT [20,47]. The non–HPLC-TNT* compounds in *E. estuarius* likely corresponded to both solvent-extractable and nonextractable compounds, as observed in other aquatic invertebrates [20,47], including the amphipod *L. plumulosus* (G. Lotufo, unpublished data). Unextractable TNT* detected in live animal tissue likely are covalently bound products associated with proteins, consistent with similar binding reported to occur in cell cultures [51,52].

The critical body residue approach for expressing toxic levels of contaminants has been used as an alternative to sediment concentration–based approaches, because it potentially improves the interpretation of toxicity by considering bioavailability, differing feeding behaviors among species, accumulation kinetics, and the effects of biotransformation (e.g., [53]). This approach assumes that the tissue concentrations of a contaminant associated with toxic effects (e.g., mortality) are similar irrespective of exposure pathways or bioavailability or compound bioavailability in the exposure media. In this study, significant mortality coincided with the highest body residues for both sediment types in TNT exposures. Critical body residues, expressed as LR50 values, were relatively similar for the exposures to SDB and YB sediments, as expected by the presence of ADNTs as the only compounds detected in the tissue solvent extracts. The LSC body residue toxicity metric values were an order of magnitude higher those calculated using HPLC data, consistent with the LC50s. The contribution of the non–HPLC-TNT* to the observed toxicity presently is unknown. Because the compounds ADNTs and DANTs are the only TNT metabolites typically assessed in environmental samples, only the HPLC-TNT* critical body residues and no-observed-effect residue/lowest-observed-effect residue reported in this study can be applied as toxicity reference values for body burden–based risk assessment of aquatic receptors.

The LSC LR50 values in this study also were higher than LSC lowest-observed-effect residues for *L. plumulosus* (38 µmol/kg), but were similar to the LSC lowest-observed-effect residue for *N. arenaceodentata* (220 µmol/kg) reported by Green et al. [13]. The HPLC LR50 values for *E. estuarius* were substantially lower than the median lethal body residues reported by Conder et al. [9] for *T. tubifex* (172 µmol/kg) and *C. tentans* (83 and 118 µmol/kg). Existing critical body residue data for HPLC-TNT* suggest that amphipods may be relatively sensitive to the lethal effects of TNT* among invertebrates.

Exposure to RDX resulted in the bioaccumulation of that compound in amphipod tissues at sublethal levels, because significant mortality was not observed in any RDX treatment. In this study, the highest mean tissue concentration measured at exposure termination (96 µmol/kg) was higher than the HPLC-TNT* body residues associated with mortality, indicating higher lethal body residues for RDX than for HPLC-TNT* for *E. estuarius*. Exposure of *L. plumulosus* to sediments spiked with radiolabeled RDX resulted in body residues of RDX molar equivalents as high as 5,960 µmol/kg [15], which did not result in significant mortality. In the latter study,

RDX parent compound was not detected by chemical analysis in tissue extracts, suggesting that the measured body burden primarily was composed of unknown degradation products. Therefore, based on the limited information on the relationship between RDX bioaccumulation and lethal toxicity, tissue concentrations as high as 96 µmol/kg in *E. estuarius* for RDX parent compound and as high as 5,960 µmol/kg sum breakdown products in *L. plumulosus* do not result in significant mortality in sediment exposures. Bioaccumulation of RDX also has been investigated in invertebrates and fish in aqueous exposures to radiolabeled RDX [52,54], but concentrations were determined using radioactivity as a surrogate for the parent compound and all its transformation products. Therefore, bioaccumulation of RDX parent compound in animals is first reported in the present study using *E. estuarius*.

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